

## Evaluating Gene Expression, Purification and Structural Characterization of the Calprotectin Subunits of S100 A8 and S100 A9

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### ABSTRACT

**Background & objectives:** Calprotectin, S100A8 and S100A9 are involved in important processes including cell signaling and regulation of inflammatory responses. In this study, recombinant expression, purification and structural characterization of S100A8 and S100A9 were accomplished.

**Methods:** In this experimental study, pET15b was used as vector of human S100A8 and S100A9 coding sequences, hosted by *E.coli* BL21 (DE3). Gene expression and purification attempts were evaluated using SDS-PAGE. Protein purification was accomplished using Ni-NTA resin based on its affinity for His-tag present on recombinant proteins. Tertiary structure of proteins were evaluated using spectrofluorimetry.

**Results:** The subunits were over expressed 3-4 hours following induction at 37 °C. S100A9 was expressed mainly as inclusion body while S100A8 was found to be expressed mainly as a soluble protein. Purification of S100A8 and S100A9 was achieved at 100 mM imidazole. Spectroscopic studies showed that the amino acid tryptophan is in the internal structures and is less exposed to the aqueous environment.

**Conclusion:** In this study, a recombinant S100A8 and S100A9 subunits were expressed and purified and also their structures were confirmed.

**Keywords:** S100A8, S100A9, *E.coli* BL21(DE3), pET15b, Ni-NTA, Spectrofluorimetry